analysis reports a synthetic lethality between mutants in which the amount of stored histone mRNA is mildly reduced and *Jabba* null mutants. Genetically reducing SLBP function by around 50% in the *Jabba* mutant is lethal, as a consequence of reduced storage of histone mRNA and possibly also reduced translation of stored histone mRNA. Similar results were obtained with a reduction in the dosage of the essential histone variant H2aV, which is also present in lipid droplets in early embryos and dependent on Jabba for storage in these organelles, presumably by forming H2b–H2aV heterodimers.

Thus, Jabba is critical for the maintenance of the pool of H2a/H2b proteins for assembly into chromatin during embryogenesis. The amount of stored histone protein in the normal egg suggests that there is little, if any, need for the synthesis of histone proteins from maternal mRNAs. However, the fact that *Jabba* mutants are viable and contain no stored histone H2a and H2b, demonstrates that there is sufficient maternal histone mRNA, including H2aV histone mRNA, to provide the histone protein necessary during embryonic development, and that these mRNAs are translated at a high rate in the absence of Jabba. It seems unlikely, although possible, that these histone mRNAs are normally translated at high rates and the histone protein rapidly degraded. It is more likely that there is cross-talk between the stored histone pool and the demand for histones in chromatin to ensure that the proper amounts of histone protein are provided, since the amount of stored histone mRNA remains constant and the demand for new histone is increasing exponentially. In the *Jabba* mutants with a slightly reduced capacity for histone synthesis, this system cannot keep up with the demand for histones H2a and H2b, resulting in death of the embryo before activation of transcription from the zygotic genome can provide additional histone mRNA.

Lipid droplets were initially thought to interact primarily with proteins involved in lipid metabolism but they are now known to be storage depots for a number of other proteins in the embryo [14], and they are bound to different sets of proteins in different cells [16]. There are reports of lipid droplets containing histones in some other proteomic studies [17], although the function, if any, of these extranuclear histones is not known. They could potentially form an additional pool of histone proteins in the cytoplasm that could be utilized during replication or DNA repair outside of S phase. Clearly lipid droplets have roles in processes other than lipid metabolism, and more of these are likely to be discovered in the future.

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Sexual Attraction: Sex-Specific Wiring of Neural Circuitry

Two recent studies describe mechanisms by which sexually dimorphic responses to pheromones in the nematode worm *Caenorhabditis elegans* are driven by differences in the balance of neural circuits that control attraction and repulsion behaviors.

E. Paxon Frady, Christopher R. Palmer, and William B. Kristan, Jr.

The question "How does the brain cause behavior" has fascinated enquiring minds ever since the brain, rather than the heart, was recognized to be the source of all behaviors [1]. The answer to the more refined question "How do neural circuits produce behavior" feels like it is getting tantalizingly close, thanks to a variety of new techniques: genomics, functional imaging, optical stimulation, multiunit arrays, microbial tracing techniques, reconstruction of serial sections, and computational

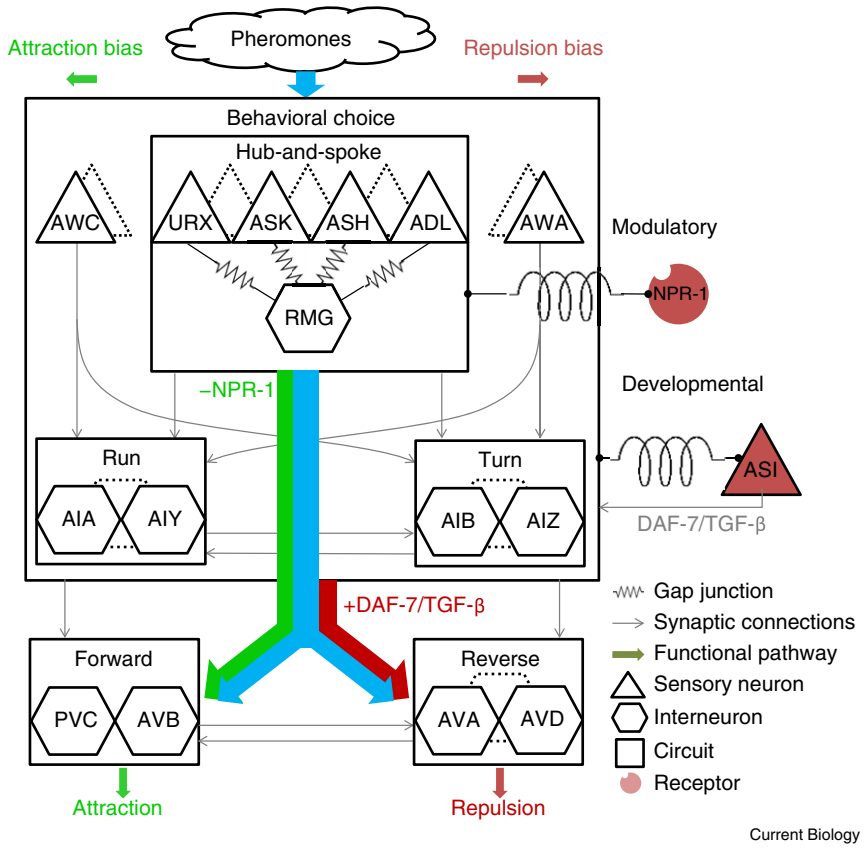

Figure 1. Attraction–repulsion behavioral choice is carried out by a circuit of chemosensory neurons and interneurons.

The functional output of this circuit is routed to forward or reverse command interneurons, which lead to attraction or repulsion behaviors. The left *versus* right positions of neurons and circuits in this diagram indicate whether the neurons produce attraction or repulsion. The springs attached to the Behavioral choice circuit and the Hub-and-spoke circuit indicate the push–pull mechanism of functional output: DAF-7/TGF-β signaling pulls the Behavioral choice circuit toward repulsion behavior, by routing more output to the reverse command neurons (red arrows), whereas knocking down *npr-1* pushes just the Hub-and-spoke circuit towards attraction (green arrows). The cell names indicated are those used in the two new papers [3,4] discussed in the text, and the dotted cell outlines indicate that there are other neurons of the same sort. The mechanisms for regulating *npr-1* are not known.

algorithms to make sense of it all [2]. Two studies [3,4] that used genetics, imaging, single-cell imaging and ablation, as well as detailed behavioral analyses in *Caenorhabditis elegans* have provided new insights into how a neuronal circuit produces behavior, and how it is modified during development [3] and by a neuromodulator [4].

Despite all the new technologies, however, the complexity of most vertebrate nervous systems is a huge barrier for exploring the role of 'microcircuitry' (cell-to-cell, rather than region-to-region, connectivity) in behavior, so looking to simpler nervous systems has proven beneficial. *C. elegans* is perhaps the simplest complete nervous system that is widely studied. Meticulous electron microscopic reconstruction, beginning with Sydney Brenner and colleagues in the 1960s, has revealed the entire 'connectome' of the hermaphrodite form of the worm [5–7]. Because knowing even parts of circuits underlying behaviors has enabled investigators to find the neuronal mechanisms underlying interactions between behaviors — such as behavioral choice [8] and context-specific variations in behaviors [9] — knowing the connectome should make finding the neuronal basis of behavioral choice and neuromodulation nearly trivial. The two new *C. elegans* studies [3,4] show that such a circuit diagram does provide a map, a high-resolution map, but complex functional studies are needed to find the street signs and to determine how the traffic moves through it.

The behavior addressed by both studies is the differential response to pheromones by *C. elegans* males and hermaphrodites (which are functionally female as breeding adults). Given the enormous overlap in the cellular components of their nervous systems — the two genders share a core set of 294 neurons that produce male-specific sexual attraction behavior [5] — any differences in behavioral response to sensory cues are likely to come from differences in how those components are wired together or how they respond to the same modulators.

The two studies [3,4] suggest that the differences in behavioral responses to pheromones result from shifts in the functional state of a finely balanced push–pull neuronal circuit. One study [3] shows how a 'behavioral choice' circuit — Figure 1, modified from a recent review of forward *versus* backward locomotion [10] — is balanced developmentally by a hormone-like transcriptional regulator (DAF-7/TGF-β). The second study [4] describes how a subset of the behavioral choice circuitry, the 'hub-and-spoke' circuit [11] (Figure 1), is tuned by a peptide receptor (NPR-1) to shift the behavioral output in the adult.

Development of the Behavioral Choice Circuitry

The developmental study [3] takes advantage of the fact that a particular chemosensory neuron (ASI) releases a wide-spectrum transcriptional regulator (DAF-7/TGF-β) that has no noticeable effect in males but suppresses attraction behavior in hermaphrodites. A series of genetic and single-cell-ablation studies showed that this suppression of the response to pheromone depends critically upon the presence of the ASI neurons. However, when ASI neurons were ablated and the regulator was expressed in other sensory neurons, behavioral suppression still occurred, indicating that the only role of ASIs in suppression, normally, was to release the regulator.

Sexually reprogramming the nervous system from hermaphrodite to male — via heatshock-induced

overexpression of a protein that degrades the master gene for sex differentiation — made pheromones attractive to the reprogrammed hermaphrodites. However, this switch was effective only if thrown at an early stage of development. Once the animal reached adulthood, reprogramming hermaphrodites to males did not make pheromones attractive. To change the attraction behavior, it was necessary to reprogram only a subset of sensory neurons (AWA, AWC, ASK) and interneurons (AIA, AIB, AIY, AIZ, RMG) that are part of the behavioral choice circuitry (Figure 1). This result indicates that the regulator DAF-7/TGF-β causes rewiring of the behavioral choice circuitry, shifting the balance towards repulsion (indicated by the spring from ASI to the behavioral circuit box in Figure 1). It is unclear whether this regulator directly disrupts the wiring amongst this subset of neurons (for example, by changing synaptic strengths) or does so indirectly (for example, by releasing neuromodulators).

Push–Pull Modulation of Behavioral Choice

In the second study [4], specific genetic manipulations were able to alter adult *C. elegans* attraction to pheromones by influencing the pheromone-sensing neurons. One sensory neuron (ADL) drives repulsion in hermaphrodites — a forward-crawling worm stops, turns, and goes in another direction — while another sensory neuron (ASK) antagonizes the ADL pathway, allowing forward progress to continue (this constitutes 'attraction' in their assay). These neurons drive the push–pull behavioral choice circuit that biases the behavior toward repulsion or attraction (Figure 1). For males, the reverse-inducing ADL responses are diminished, pulling the balance towards attraction, whereas for hermaphrodites, the balance favors repulsion.

A neuropeptide receptor, NPR-1, influences the behavioral choice acutely [4], rather than developmentally, because turning-on the *npr-1* gene through heatshock alters the behavior of adult worms within minutes. This observation suggests that the behavioral choice circuitry is biased by a neuromodulatory pathway that activates the neuropeptide receptor NPR-1 (indicated by the right-hand spring to the Hub-and-spoke circuit in Figure 1). This receptor influences the behavioral choice circuit through interneuron RMG, a cell that has gap-junction connections to a large set of sensory and interneurons, forming a 'hub-and-spoke circuit' [11]. This circuit appears to be a target of NPR-1, because blocking RMG's gap junctions erases the effects of activating NPR-1, and mutating *npr*-1 potentiates ASK signaling and inhibits ADL signaling — shifting the balance towards attraction. Although the detailed mechanism is not yet known, a reasonable hypothesis is that NPR-1 closes gap junctions, which could potentially disengage the entire hub-and-spoke circuit [4].

Cell RMG, the 'hub' neuron, may play a key role in driving *C. elegans* locomotory behavior. A major component of the *C. elegans* locomotory behavior is a biased random walk [12], also called klinokinesis [10]. This model is a balance between 'runs' (straight-ahead trajectories) and 'pirouettes' (randomly directed turns). When a healthy, well-fed male happens to be moving up a pheromone concentration gradient, its locomotor circuitry is pushed toward the 'straight-ahead' state. This increases excitation to forward command neurons, which keep the animal moving forward. If, however, the same male happens on the pheromone trail moving down the pheromone gradient, the circuitry is pulled toward the 'random' state, causing the animal to change directions — pirouette — and explore for new cues. The neuromodulator that activates NPR-1 may be the way that context (for example, internal state or external stimuli) alters the balance between forward progress and random states, which cause the animal to be attracted or repulsed by different sensory cues (Figure 1). In fact, there is an additional, small bias up the chemical gradient during both the runs (which are not straight but bent [13]) and the pirouettes (which more often end up heading up the gradient than predicted by pure chance [12]); both biases help to orient the searching male more quickly up the chemical gradient.

Why Is a Complete Connectome Not Enough?

These two papers [3,4] are at the same time elegant and humbling. One might think that, with such a simple nervous system and a complete circuit diagram, one could simply look at the connectome and figure out which neurons are modified by the genes and modulators. Why have two incredibly intensive studies come to such tentative conclusions about the site of a neuromodulator and a developmental regulator? The answer is that, although the anatomical connectome gives a huge amount of information, it does not provide information about four important features: the polarity of electrical connections (rectifying or non-rectifying); the nature of chemical synapses (excitatory or inhibitory); synaptic strength; and neuronal dynamics (both of cellular properties and of the system as a whole). In fact, the system appears to be overly connected: neurons make so many connections that many of them must be ignored to propose any kind of circuit that can produce a sensible behavioral output.

One possible explanation for this super-connectivity is that the connections cover the range of possibilities for different circuits, and that neuromodulators activate selected parts of the anatomical network [14,15]. Another, non-exclusive, possibility is that the same network can have multiple functional stable states that arise through the dynamics of the system, so that the same neurons can perform different behaviors depending on their relative timing [16,17]. Whatever the ultimate mechanism, these two impressive studies on the nematode nicely bring home the message that we must be plastic in how we think about neuronal circuits, which are clearly more dynamic and fluid than can be captured by a circuit diagram.

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Evolution: Cichlid Models on the Runaway to Speciation

Rapid speciation has fascinated biologists for a long time. A recent study shows that ecological opportunity and sex-biased color differences increase the likelihood of speciation in African cichlid fishes.

**Hugo F. Gante*
and Walter Salzburger**

Speciation, the origin of novel species, is a complex and multilayered process that has remained hard to understand for empiricists and theoreticians alike. Researchers have dedicated much effort to pinpointing the factors and conditions that are responsible for some taxa diversifying rapidly while others linger in a speciation stasis. Only now are we realizing that it is the coupling of different intrinsic (e.g. natural history, genetics) and extrinsic (e.g. climate, habitat, behavioral interference) factors that produces the speciation momentum of adaptive radiations [1,2]. During adaptive radiations, a typically generalist ancestor diversifies in a short period of time into multiple specialized species that then occupy novel ecological niches. Famous examples include Darwin's finches in the Galápagos archipelago and the Caribbean *Anolis* lizards. But arguably the most spectacular radiations among vertebrates are those of African cichlid fishes inhabiting the three African Great Lakes — Malawi, Victoria and Tanganyika (Figure 1). The independent adaptive radiations of cichlid fishes in these lakes have produced a great number of species — estimates point to over 1500 — the vast majority of which are endemic to each lake and differ in their pigmentation patterns, body shapes, and reproductive and social behaviors [1]. Interestingly, several extant cichlid lineages did not diversify explosively. Radiating and non-radiating lineages can be found not only in the East African Great Lakes, but also in dozens of other smaller African lakes inhabited by distinct cichlid assemblages. This naturally widespread system of closely related species provides the perfect setting for evaluating which intrinsic and extrinsic attributes account for some lineages, but not others, having undergone adaptive radiations [1].

This was exactly what Wagner *et al.* [2] set out to do in a recent paper: in an elegant continent-wide study, the authors compiled data on colonization and diversification of African cichlids in 46 lakes. Physical and environmental data for each lake (e.g. age, depth, net solar radiation) and lineage-specific traits (e.g. mating systems, brooding of eggs and fry in the mouth, sexual differences in pigmentation) that could potentially explain diversification were contrasted using phylogenetic logistic and hurdle Poisson regressions. These comparative methods assess the association between predictor extrinsic and intrinsic variables, and whether or not a lineage diversified in a lake.

Wagner *et al.* [2] found that environmental conditions increasing ecological opportunity in deeper lakes with higher solar energy input, together with high levels of sexual dichromatism (sex-biased differences in pigmentation), predict an increased likelihood of cichlid diversification. Importantly, cichlid diversification is best explained by the combined effects of extrinsic environmental variables and intrinsic lineage-specific traits. This explains differences in diversification rates between lakes and why only some lineages diversify in a subset of the lakes inhabited by cichlids. Deeper lakes with higher energy input are probably more stable over evolutionary times. They also have a greater number of ecological niches, which are more productive and more diverse, and are overall able to sustain larger numbers of individuals (higher carrying capacity). Together, these environmental factors increase the ecological opportunity for cichlids and allow them to radiate.

In addition, radiations are more likely to occur in sexually dichromatic lineages, which also explains differences in speciation rates among lineages within lakes. High sexual dichromatism is commonly interpreted as evidence for strong sexual selection. In cichlids, sexual dichromatism has evolved only in species with polygamous mating systems, in which females are choosy and select among males based on their eye-catching pigmentation, while males mate with as many females as possible. Therefore,